

## *Communications*

# Corneal granular dystrophy

## A light and electron microscopical study of its recurrence in a graft

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Histochemical evidence suggests that the amorphous stromal deposits of corneal granular dystrophy are composed essentially of a non-collagenous protein or polypeptide complex, the precise nature of which, however, is unknown (Seitz and Goslar, 1963; Garner, 1969). Electron microscopy fortunately enables a further dimension of this material to be studied, and the present report gives the findings in a corneal disc removed from an established case of granular dystrophy during a repeat keratoplasty after the recurrence of the disorder in the original graft.

### **Material and methods**

Two specimens were available: the first was a full-thickness disc obtained when the patient, a female aged 33 years, was subjected to penetrating keratoplasty of the right eye, and the second was a partial-thickness disc removed some 10 years later from the same eye after the development of superficially-placed opacities in the donor tissue.

The original specimen had been fixed in 10 per cent. formol-saline and embedded in paraffin wax for histological sectioning at the Queen Victoria Hospital, East Grinstead; the second was received in buffered glutaraldehyde at the Institute of Ophthalmology, London, where it was divided into two parts, for light and electron microscope study respectively. The portion selected for electron microscopy was post-fixed in Zetterqvist's osmium tetroxide and embedded in Araldite, and selected areas were sectioned at 500 to 800 Å on an LKB ultratome III. Sections, stained with uranyl acetate followed by either lead citrate or phosphotungstic acid, were then examined with an AEI-EM6 electron microscope.

The remaining portion of the disc was processed for light microscopy, sectioned at a thickness of 5 µ, and, in addition to routine staining, subjected to specific staining for protein and amino-acid groups as previously described (Garner, 1969). Sections from the original specimen were similarly stained.

### **Results**

#### **LIGHT MICROSCOPY**

The tissue removed at the time of the first graft operation consisted of a full-thickness disc of cornea from which the surface epithelium had been artificially detached. Bowman's membrane was completely destroyed and the stroma at all levels included widespread focal deposits of an amorphous eosinophilic material. Descemet's membrane was intact and

there was no apparent endothelial abnormality. The stromal deposits stained red by the Masson trichrome procedure (Fig. 1), but gave no reaction with either periodic acid-Schiff or acid polysaccharide staining methods; they gave positive reactions for protein and were shown to contain tyrosine, arginine, tryptophan, and sulphur-containing amino-acids.

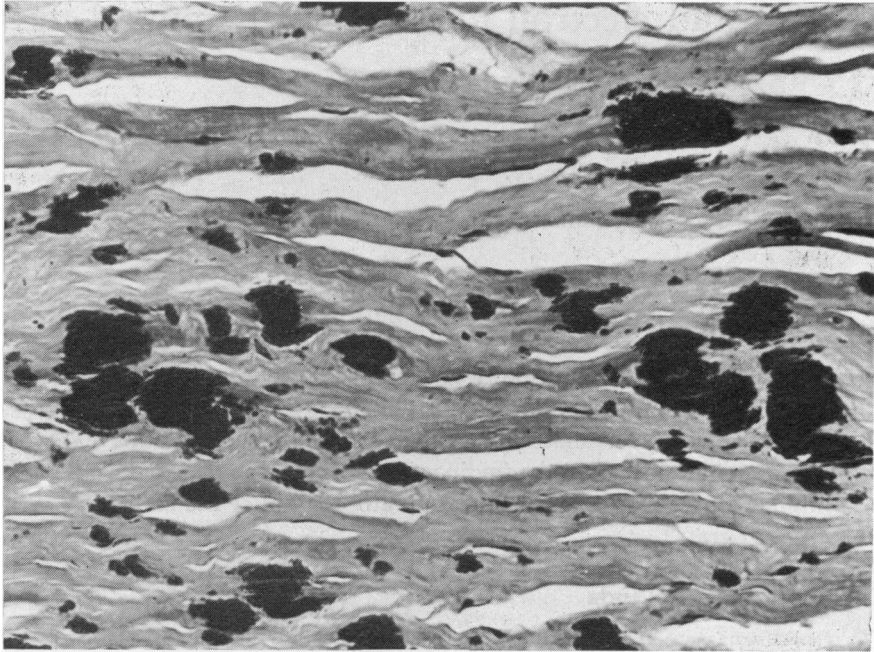


FIG. 1 Section of corneal disc removed during initial graft operation, showing red-staining granular deposits distributed throughout the substantia propria. Masson trichrome  $\times 340$

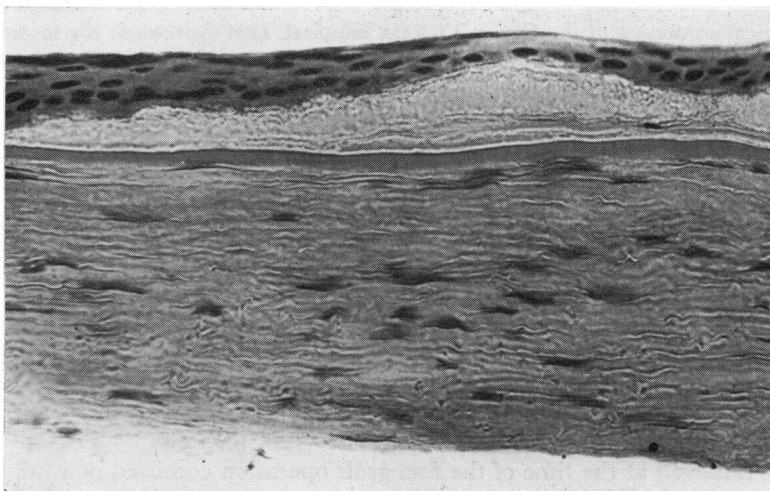


FIG. 2 Lamellar disc of donor cornea removed at second keratoplasty, showing accumulation of granular material between Bowman's membrane and the epithelium. Haematoxylin and eosin  $\times 225$

The lamellar disc removed at the second operation was made up mainly of donor tissue with a small margin of host cornea at the periphery. The stroma of the donor cornea was apparently normal and Bowman's membrane was intact, but between the latter and the epithelium there was a layer of amorphous material (Figs 2 to 4) comparable to that seen in the stroma of the original specimen. Similar deposits were present in the stroma of the host tissue present at the edges of the donor tissue. Apart from an essentially negative response to Masson's trichrome stain, a finding almost certainly due to prolonged fixation in glutaraldehyde, the staining reactions of the tissue deposits in the second specimen were identical to those in the first. Amyloid was not demonstrable in either.

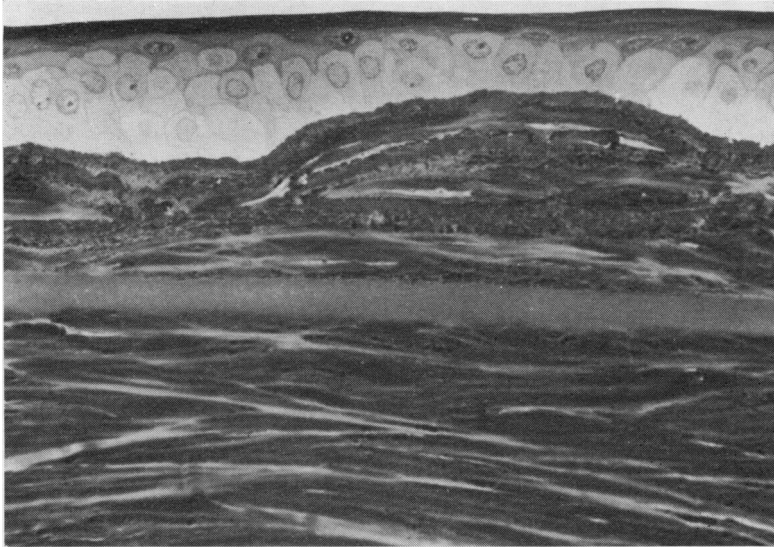


FIG. 3 *Araldite section of the recurrence, showing granular material between Bowman's membrane and the epithelium deposited within a collagenous matrix. Toluidine blue  $\times 360$*

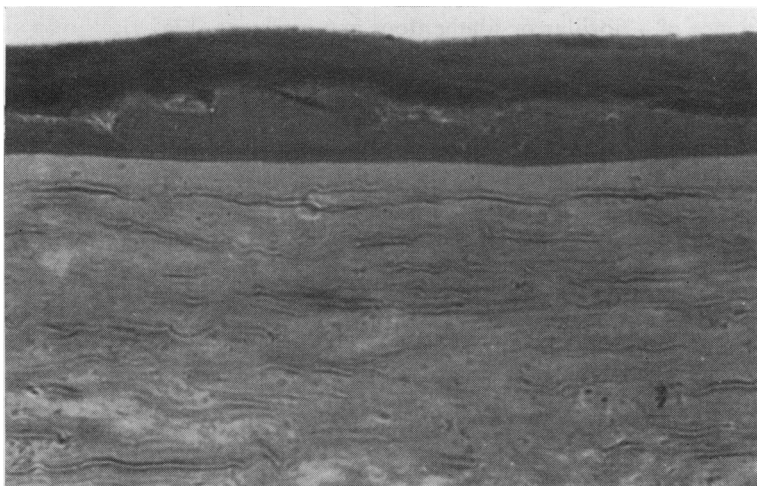


FIG. 4 *Subepithelial material stains strongly for protein, as also does the overlying epithelium; the stroma and Bowman's membrane are less intensely stained. Coupled tetrazonium  $\times 225$*

Details of the staining reactions in the two specimens are presented in the Table.

**Table** *Histological staining reactions of the corneal lesions*

| <i>Method</i>  | <i>Reaction of granular deposits</i> |                       |
|--|--------------------------------------|-----------------------|
|  | <i>Original specimen</i>             | <i>Recurrence</i>     |
| Haematoxylin and eosin                                       | Eosinophilic                         | Eosinophilic (uneven) |
| Masson trichrome   | Red                                  | Unstained             |
| Wilder silver  | +                                    | +                     |
| Birefringence  | —                                    | —                     |
| Periodic acid-Schiff   | —                                    | —                     |
| Alcian blue (pH 2.5)   | —                                    | —                     |
| Congo red  | —                                    | —                     |
| Methyl violet  | Orthochromatic                       | Orthochromatic        |
| Thioflavine-T fluorescence                                   | —                                    | —                     |
| Coupled tetrazonium for protein                              | ++                                   | ++                    |
| Ninhydrin-Schiff for protein-bound amino groups              | —                                    | —                     |
| Hydroxynaphthaldehyde for protein-bound amino groups         | +                                    | ±                     |
| Diazotization-coupling for tyrosine                          | ++                                   | +                     |
| p-Dimethylaminobenzaldehyde for tryptophan                   | ±                                    | ±                     |
| Sakaguchi for arginine                                       | +                                    | +                     |
| Dihydroxy-dinaphthyl-disulphide (DDD) for sulphhydryl groups | +                                    | ++                    |
| Thioglycollate—DDD for sulphhydryl and disulphide groups     | ++                                   | ++                    |

#### ELECTRON MICROSCOPY

The most striking feature was the presence, between Bowman's membrane and the epithelium of the donor cornea, of electron dense structures which at low resolution had a superficially crystalline appearance (Figs 5 and 6). Presenting as an agglomeration of variably sized rod-shaped or trapezoid profiles, and ranging in width from 100 to 500 mμ, the deposits were shown at higher magnification to be composed of an amorphous material without evidence of a fibrillar or filamentous arrangement (Fig. 7).

Between the deposits there was a network of randomly orientated collagen fibrils together with some finer filaments (Fig. 8) such as are commonly found in corneal scar tissue in a variety of conditions (Rice, Ashton, Jay, and Blach, 1968), but apart from their spatial proximity there was nothing to suggest that the amorphous deposits were a product of these collagenous elements. Associated with the collagen fibrils was a small number of fibroblasts, the majority of which appeared entirely normal (Fig. 9), although occasional cells showed some vacuolation of their cytoplasm.

The overlying epithelium was apparently normal, showing no evidence of secretory activity or of degeneration (Fig. 10), while both Bowman's membrane and the underlying superficial lamellae of the substantia propria were composed of mature collagen with complete absence of the abnormal deposits present in the subepithelial zone.

#### Discussion

Of the few published reports of the ultrastructure of corneal granular dystrophy (McTigue, 1965, 1967; Sornson, 1965; Kuwahara, Akiya, and Obazawa, 1967; Matsuo, Fujiwara and Ofuchi, 1967; Teng, 1967), only Matsuo and his colleagues present findings analogous to



FIG. 5 Survey electron micrograph of recurrence, showing subepithelial deposition of electron dense trapezoid and rod-shaped "crystals". Uranyl acetate-lead citrate  $\times 10,000$

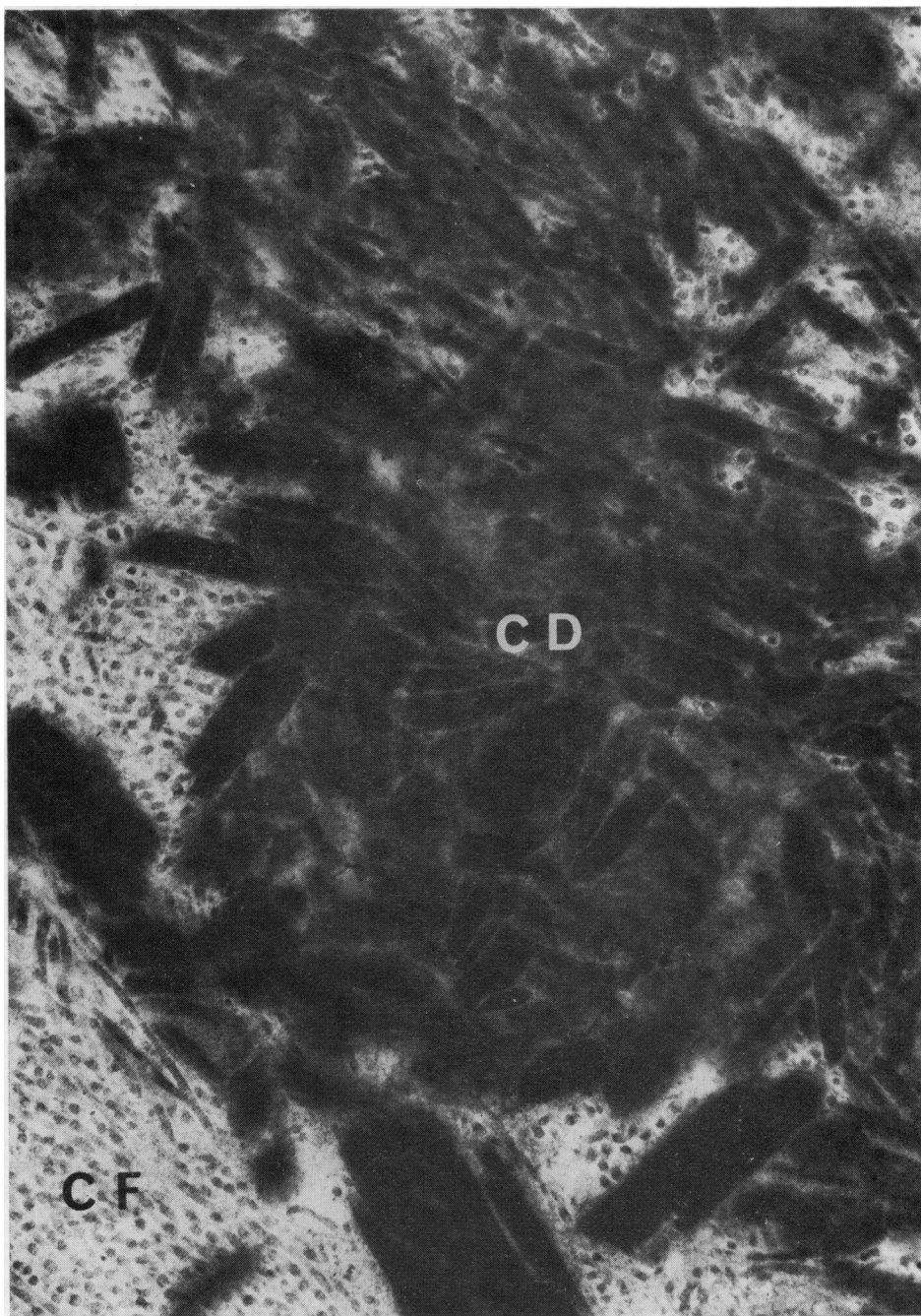


FIG. 6 "Crystalline" deposits (CD) lie within a matrix of randomly orientated mature collagen fibrils (CF) Uranyl acetate-lead citrate. Electron micrograph  $\times 50,000$



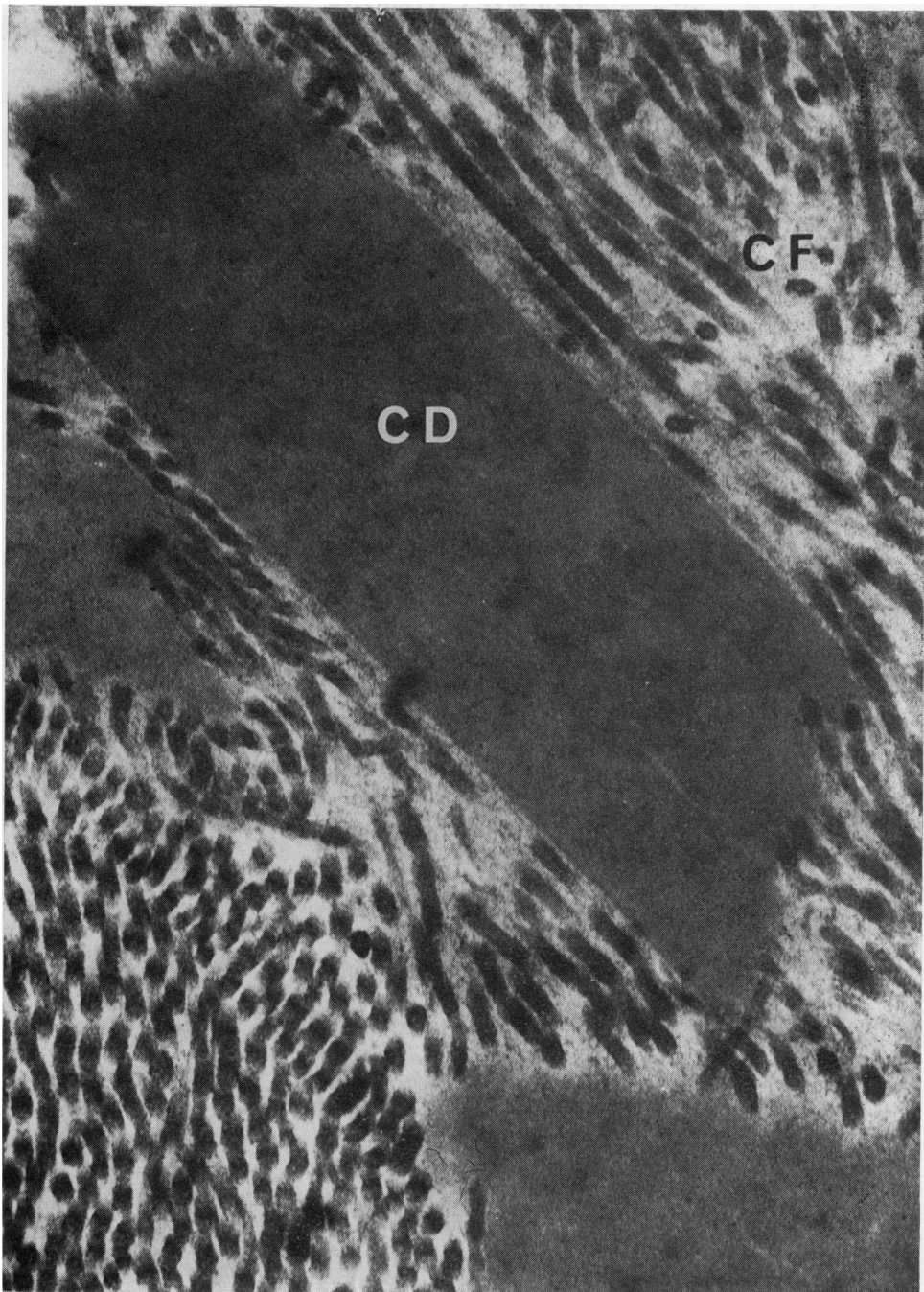


FIG. 7 At higher magnification the "crystalline" deposits (CD), seen here as a trapezoid structure, appear to be composed of a homogeneous amorphous material. CF, collagen fibrils. Uranyl acetate-phosphotungstic acid. Electron micrograph  $\times 97,500$

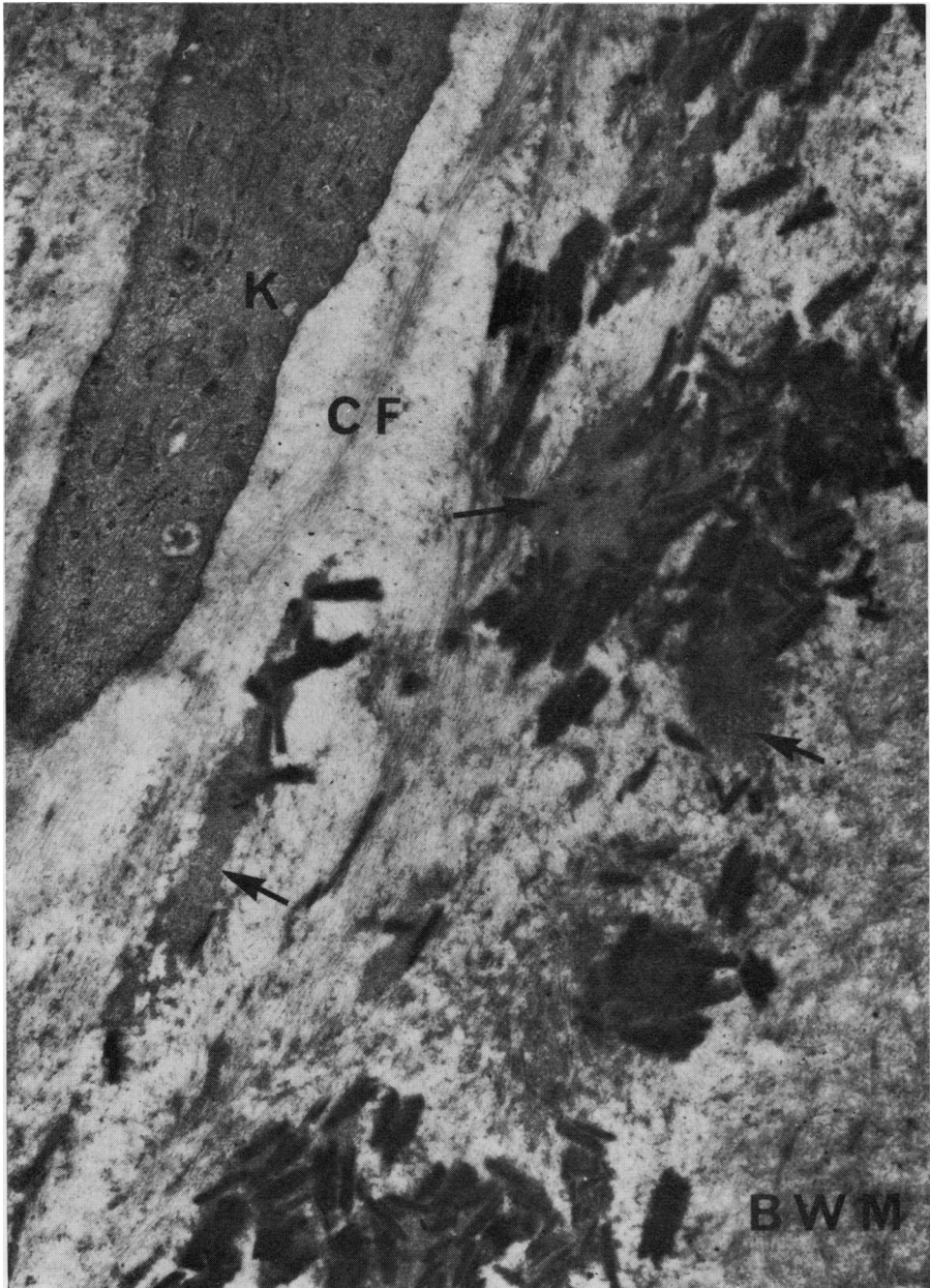


FIG. 8 In addition to mature collagen fibrils (CF), the tissue around the "crystalline" deposits includes areas of moderate electron density (arrowed) which are composed of fine filaments grouped together in irregular bundles. The cytoplasm of an adjacent keratocyte (K) shows no abnormality. BWM, Bowman's membrane. Uranyl acetate-lead citrate. Electron micrograph  $\times 20,000$



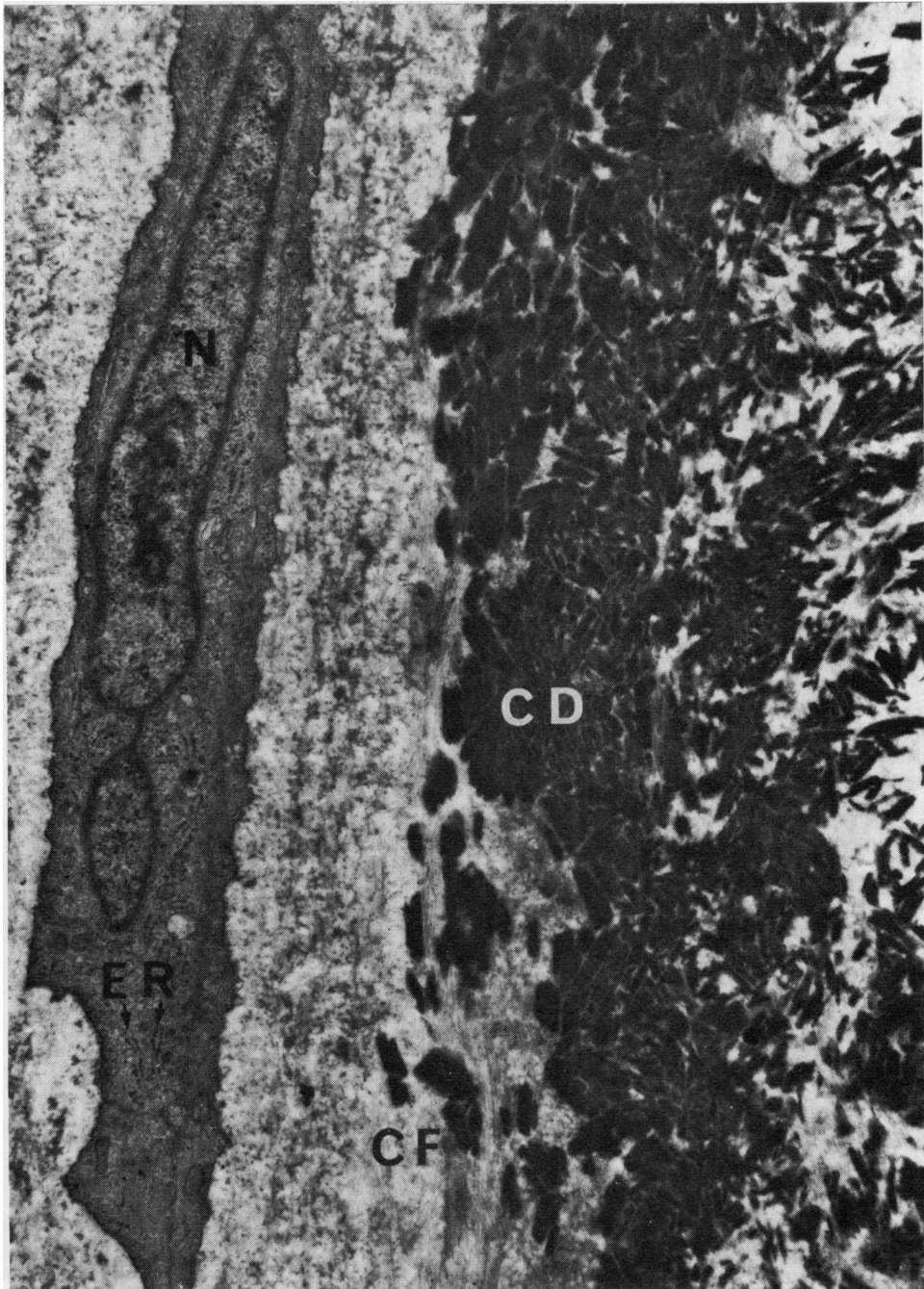


FIG. 9 Most of the keratocytes associated with the "crystalline" deposits (CD) showed no obvious sign of degenerative change. N, nucleus; ER, endoplasmic reticulum; CF, collagen fibrils. Uranyl acetate-lead citrate. Electron micrograph  $\times 15,000$



FIG. 10 Survey electron micrograph, showing that while the "crystalline" deposits (CD) abut on to the basement membrane (BM) of the corneal epithelium, the cells themselves appear normal. Uranyl acetate-lead citrate. Approx.  $\times 2,300$

our own. (Kuwahara's group comment on the presence of rod-shaped structures but do not provide any pictorial evidence, so that their contribution cannot be assessed.) Nevertheless, there would seem to be good reason to regard these "crystalline" structures as characteristic of this disorder, since study of another case (Case 1 of the series reported by Garner, 1969), in which the cornea had previously been embedded in paraffin wax for histological examination, has shown the same type of deposit within the stroma (Fig. 11).

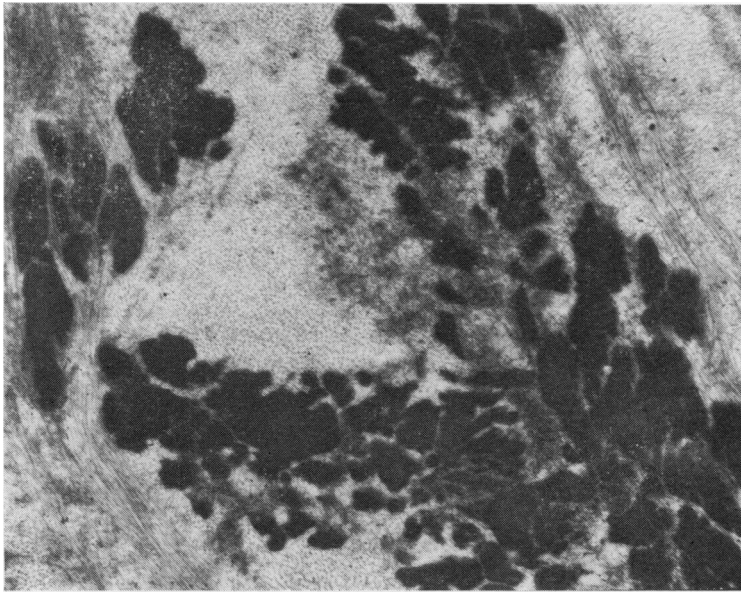


FIG. 11 *Electron micrograph of stromal deposits in typical granular dystrophy. The material was obtained by de-paraffinizing tissue previously embedded for histological examination. Despite inadequate processing for electron microscopy, the "crystalline" nature of the deposits is still readily seen. Formol-saline fixation. Post-fixed in osmium tetroxide. Uranyl acetate-lead citrate.  $\times 19,500$*

On the basis of positive congo red staining, Matsuo and others (1967) interpreted these structures as being amyloid but, since this reaction can be regarded as diagnostic only if the deposits so stained exhibit green dichroism when viewed between crossed Nicol prisms (Missmahl, 1957), their conclusion is not necessarily valid, especially as there is no record that this step was carried out. Furthermore, in neither their study nor ours did the electron microscopic appearances of the deposits bear any resemblance to the characteristic fibrils described in other forms of amyloidosis (Cohen, 1967). A non-fibrillar component or precursor of amyloid has recently been described, however, which might be expected, at electron microscope resolutions comparable to those obtainable in our study, to present as an amorphous or finely granular moiety (Bladen, Nylén, and Glenner, 1956; Hirschl, 1969), and the possibility that the tissue deposits in granular dystrophy may be related in some way to this component cannot therefore be discounted.

While the histochemical and ultrastructural evidence in the present case has failed to identify the material in question, it cannot exclude the possibility that it may be related to amyloid, but it is strongly against an origin from collagen, either as a degenerative product (Franceschetti and Babel, 1951) or as an incomplete precursor (Teng, 1967). The composition of collagen, and for that matter of reticulin, is incompatible with the demonstrable amino-acid content of the tissue deposits in granular dystrophy, as has already been observed (Garner, 1969). Moreover, the ultrastructure of the deposits is quite unlike that described for immature collagen or reticulin (Melcher, 1966). That reticulin or some related incomplete form of collagen is also present is not denied. Indeed the study by Teng (1967) showing large numbers of fine filaments within the lesions, our own finding of fine fibrils similar to those seen in scar tissue occurring in other forms of corneal disease

(Rice and others, 1968), and the demonstration of argyrophilic fibres by light microscopy make it extremely likely. It is, however, our impression that these components which are probably non-specific are of secondary importance in the pathogenesis of the disorder.

On account of some histochemical resemblance to keratin or keratohyaline, the suggestion has been made that the granular deposits might possibly derive from abnormal squamous epithelium (Garner, 1969), but this finds no support in the present study. Rather the absence of any discernible epithelial abnormality in the donor corneal graft points to the deposits being a product of host keratocytes which had invaded the sub-epithelial zone to form an anterior graft membrane of the type not uncommonly seen in unsuccessful keratoplasties (Winter, 1969). It is to be noted that only a minority of the keratocytes situated in and around the subepithelial deposits showed any sign of degenerative change, which suggests that, while cellular degeneration may occur as Sornson (1965) and Teng (1967) have stressed, it is probably not essential for the elaboration of the granular material.

### Summary

Following the recurrence of opacities in the grafted cornea in the eye of a female patient suffering from hereditary granular dystrophy, a repeat keratoplasty was performed and the excised lamellar disc of opaque tissue was submitted for histochemical and electron microscopical study. This revealed accumulations of a protein substance between Bowman's membrane and the epithelium which ultrastructurally presented as amorphous material, grouped in bizarre rod-shaped and trapezoid masses, and deposited within a collagenous environment. Its possible nature is discussed.

It is a pleasure to acknowledge our indebtedness to Mr. A. Werb of the Corneo-Plastic Unit at the Queen Victoria Hospital, East Grinstead, for allowing us to publish this case, and to Major-General A. Sachs for kindly providing material from the original corneal specimen. We would also thank Prof. N. Ashton for his invaluable comments and advice, Mr. A. McNeil and Miss Susan Legg for technical assistance, and Miss Ruth Mason for secretarial assistance.

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